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Oogenesis and Reproductive Cycle in *Ruditapes philippinarum* on the West Coast of Korea

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ABSTRACT

Oogenesis and the reproductive cycle in female *Ruditapes philippinarum* were investigated by cytological and histological observations. *R. philippinarum* is dioecious and oviparous. During vitellogenesis, the Golgi complex, glycogen particles and mitochondria were involved in the formation of lipid droplets and lipid granules in the cytoplasm of the early vitellogenic oocyte. In the late vitellogenic oocyte, cortical granules, the endoplasmic reticulum, and mitochondria were involved in the formation of proteid yolk granules in the cytoplasm. At this time, exogenous lipid granular substance and glycogen particles in the germinal epithelium passed into the oocyte through the microvilli of the vitelline envelope. The spawning period was once a year between early June and early October, and the main spawning occurred between July and August when seawater temperature was approximately 20℃. The reproductive cycle of this species can be categorized into five successive stages: early active stage (January to March), late active stage (February to May), ripe stage (April to August), partially spawned stage (May to October), and spent/inactive stage (August to February). Percentages of female clams at first sexual maturity of 15.1-20.0 mm in shell length were 52.6% (50% of the rate of group maturity was 17.83 mm in length), and 100% for the clams > 25.1 mm.

Keywords: *Ruditapes philippinarum*, Oogenesis, Reproductive Cycle.

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INTRODUCTION

Ruditapes philippinarum (Bivalvia: Veneridae) is widely distributed along the coasts of Korea, China, Japan, the United States, and Spain, *etc.* It is particularly found in the intertidal and subtidal zones of the south and west coasts of Korea (Yoo, 1976; Kwon *et al.,* 1993; Chung *et al.,* 1994). In Korea, this species is one of the most important marine resources for human consumption. Standing stock of this commercially important clam has been declining for decades due to marine reclamation project of tidal areas marine pollution, and reckless overharvesting. Therefore, it is necessary to manage the population of this clam using a proper catching regime and detailed information that will maintain optimal population size in shellfish farm.

So far, many studies have examined various aspects of the reproductive ecology of *Ruditapes philippinarum* in Korea, Japan and the other countries (Hur, 1994), population dynamics and secondary production (Ohba, 1959; Choi, 1987; Yoon, 1992), reproduction including maturation (Toba and Miyama, 1995), artificial discharge (Sagara, 1958), the spawning season (Yoshida, 1953; Tanaka, 1954; Ohba, 1959; Holland and Chew, 1974; Ponurovsky and Yakovlev, 1992; Chung *et al.,* 1994), and the reproductive cycle (Toba *et al.,* 1993; Toba and Miyama, 1994; Chung *et al.,* 1994; Tsuji, 1994; Goshima *et al.,* 1996). Nevertheless, no information is available for reproductive mechanism of vitellogenesis

during oogenesis of this clam. Better understandings of the reproductive cycle and spawning period of this species need to determining population age structure and recruitment period. In addition, data for first sexual maturity and prohibitory measure of the population would be very useful information for aquaculture and the management of natural resources.

The present study provides some information on the reproductive mechanism of vitellogenesis during oogenesis and reproductive ecological data of *Ruditapes philippinarum* including some basic information on the ovarian development, first sexual maturity and prohibitory measure for propagation and management.

MATERIALS AND METHODS

1. Sampling

Specimens of *Ruditapes philippinarum* were collected monthly at the intertidal zone of Gomso Bay, west coast of Korea, for two years from January 2004 to December 2005 (Fig. 1). Female clams ranging from 8.4 mm to 54.6 mm in shell length were used for the cytological and histological studies. After the alive clams were transported to the laboratory, shell length and height were measured by a Vernier caliper, and total weight was measured using a top-loading electronic balance (Casbee MW-120).

2. Germ cell differentiation during oogenesis by electron microscopic observation

For electron microscopic observation, excised pieces of ovaries were cut into small pieces and fixed immediately in 2.5% paraformaldehyde prefixation, in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4℃ After prefixation, the specimens were washed several time in the buffer solution and then postfixed in 1% osmium tetroxide solution in 0.2 M phosphate buffer solution (pH 7.4) at 4℃ for 1 hour.

Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and a LKB Ultramicrotome at a thickness of about 800-1000 Å. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrated, and observed with a JEM 100CX-2 (80 kV) electron microscope.

3. Ovarian maturation by histological observations

Histological preparations of the ovaries were made for analysis of the ovarian developmental phases by light microscopy. A total of 167 female clams over 15.1 mm in shell length were used for the histological study. Tissues were removed from shells and preserved in Bouin's fixative for 24 hours and then

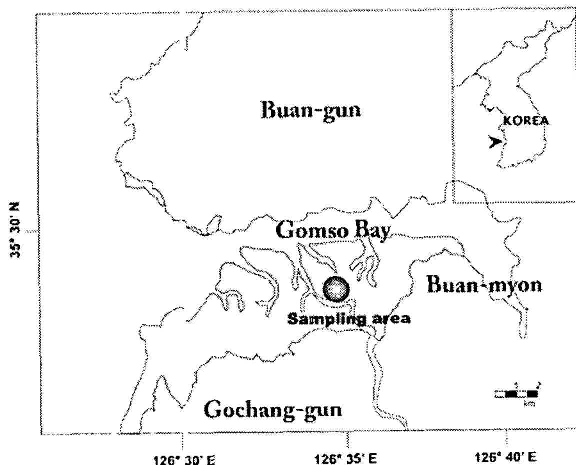

Fig. 1. Map showing the sampling area.

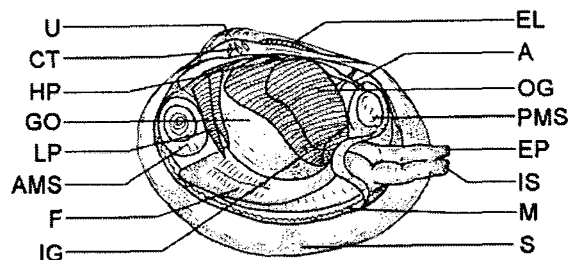

Fig. 2. Anatomy of *Ruditapes philippinarum*.
Abbreviations: A, anus; AMS, anterior adductor muscle scar; CT, cardinal tooth; EL, external ligament; ES, exhalant siphon; F, foot; GO, gonad; H, hepatopancreas; IG, inner gill; IS, incurrent siphon; LP, labial palp; M, mantle; OG, outer gill; PMS, posterior adductor muscle scar; U, umbo.

washed with running tap water for 24 hours. The tissues were then dehydrated in alcohol, embedded in paraffin and sectioned at 5-7 μm using a rotary microtome. Sections were then mounted on glass slides, stained with either Hansen's hematoxylin-0.5% eosin, Mallory's triple stain or PAS stain, and were analyzed using a light microscope. Examination of ovary variability in *Ruditapes philippinarum* showed no significant differences in reproductive state between seven random sections taken from different positions in the ovary. Sections were assigned to one of five stages: 1) early active stage, 2) late active stage, 3) ripe stage, 4) partially spawned stage, and 5) spent/inactive stage, based on modifications of the staging criteria used by Redfern (1974). Two or more stages often occurred simultaneously within each section, therefore, the staging criteria decisions were based upon the conditions of the majority of the section.

RESULTS

1. Position and structure of the ovary

Ruditapes philippinarum is dioecious. The ovary is located between the subregion of the midintestinal glands in the visceral cavity and the reticular connective tissues of the foot (Fig. 2). The ovary is composed of a number of oogenic follicles.

As maturation progresses, the ovary extended to the reticular connective tissue of the foot. At this time, external view of the ovary is light pink in colour. But after spawning, ovaries rapidly degenerated, and then the area of the ovary decreased among the digestive diverticula, muscle tissue.

2. Germ cell differentiation during oogenesis by electron microscopic observation

Oogenesis occurs in the oogenic follicles of the ovary and can be divided into four phases: (1) premeiotic phase, (2) previtellogenic phase, (3) vitellogenic phase, and (4) mature phase.

Premeiotic phase: The stem cells, which constituted the boundaries of the follicle, gave rise to primary oogonia (approximately 9-10 μm.), characterized by a high nuclear-cytoplasmic ratio, and the primary oogonia were divided mitotically to produce the secondary oogonia (Fig. 3A). At this phase, granular cells (containing a number of granules and mitochondria) and undifferentiated mesenchymal cells were present around the oogonia. Undifferentiated mesenchymal cells contained long and irregular nucleus with chromatin nucleolus, and have several mitochondria and several vacuoles in the cytoplasm (Fig. 3B).

Previtellogenic phase: As the oogonium enter into the first prophase of meiosis, oogonia developed into the previtellogenic oocytes. At the beginning of cytoplasmic growth, the nucleus and cytoplasm of the previtellogenic oocyte increased in volume at this phase, the nucleus and oocyte diameters were 4-5 μm and 15-25 μm, respectively. A number of mitochondria and endoplasmic reticulum in the cytoplasm were concentrated around the nucleus. But at this phase, the microvilli on the vitelline envelope of the oocyte were not present (Fig. 3C).

Vitellogenic phase: As the development of previtellogenic oocytes proceed, the oocytes entered into vitellogenesis in the early vitellogenic phase. The early vitellogenic oocytes further enlarged to 30-40 μm in diameter, and the Golgi complex was present in the perinuclear region of the cytoplasm, and numerous vacuoles and vesicles were scattered from the perinuclear region to the vitelline envelope of the oocyte. Lipid droplets were present in the vacuoles and vesicles which were formed by the Golgi complex in the perinuclear cytoplasm and were dispersed toward the cortical layer near the vitelline envelope (Fig. 3D).

On the other hand, lipid droplets appeared among the mitochondria, well-developed rough endoplasmic reticula and glycogen particles near the nucleus (Fig. 3E). At this phase the microvilli on the vitelline envelope appeared, and the contours of the microvilli were round or oval in shape. Round cortical granules began to appear among lipid droplets, lipid granules, mitochondria and glycogen particles in the cortical

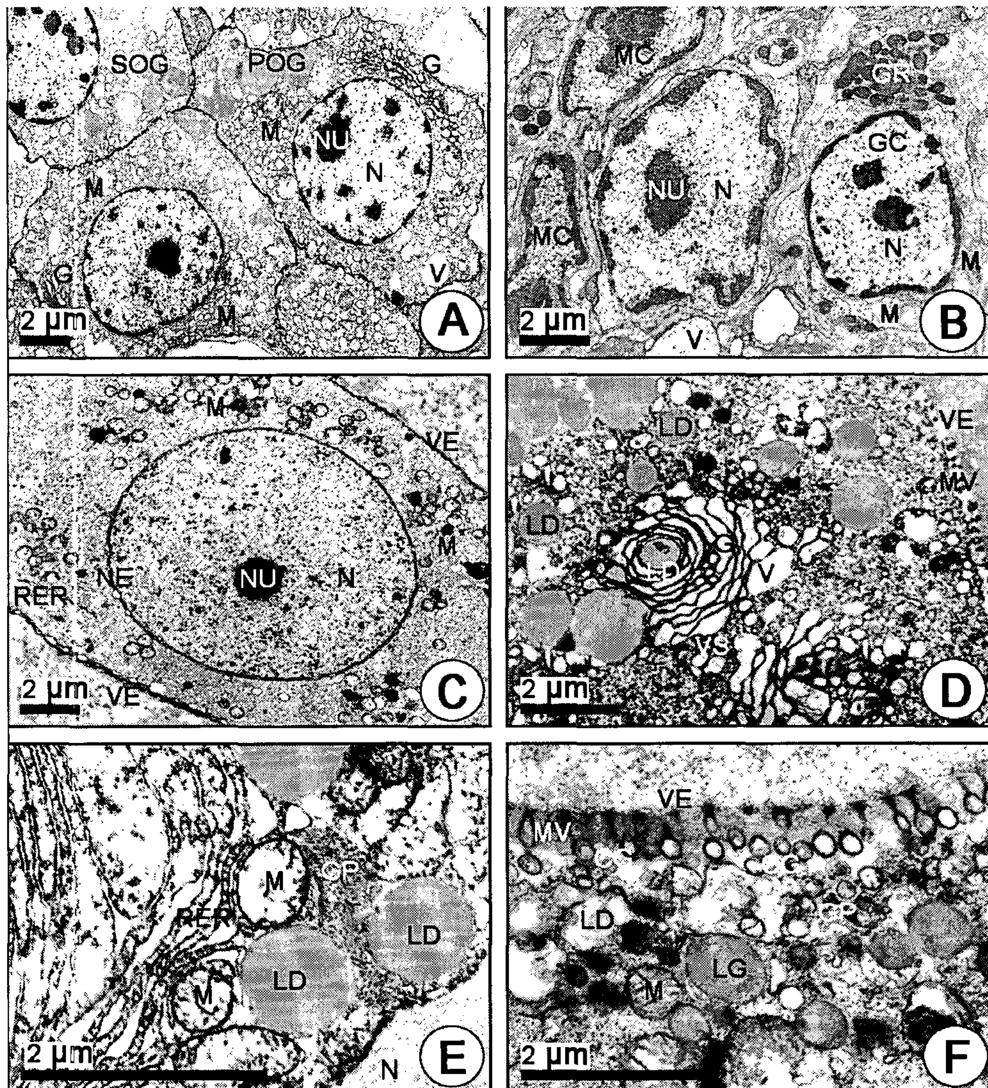

Fig. 3. Electron micrographs of the previtellogenic and early vitellogenic oocytes during oogenesis of *Ruditapes philippinarum* (A-F). **A**. Oogonia, with a large nucleus, several mitochondria and the Golgi complex in the cytoplasm. **B**. Undifferentiated mesenchymal cells and the granular cells near the oogonia. **C**. A previtellogenic oocyte, with a large nucleus, the endoplasmic reticulum and several mitochondria near the vitelline envelope. **D**. An early vitellogenic oocyte, a number of lipid droplets in the vacuoles and vesicles near the Golgi complex. **E**. An early vitellogenic oocyte, with lipid droplets which surrounded by the mitochondria, rough endoplasmic reticula and glycogen particles. **F**. An early vitellogenic oocyte, with the cortical granules, lipid droplets and lipid granules near the vitelline envelope.

Abbreviations: CG, cortical granule; G, Golgi complex; GC, granular cell; GP, glycogen particle; GR, granule; LD, lipid droplet; LG, lipid granule; M, mitochondrion; MC, mesechymal cell; MV, microvilli; N, nucleus; NE, nuclear envelope; NU, nucleolus; POG, primary oogonium; RER, rough endoplasmic reticulum; SOG, secondary oogonium; V, vacuole; VE, vitelline envelope; VS, vesicle.

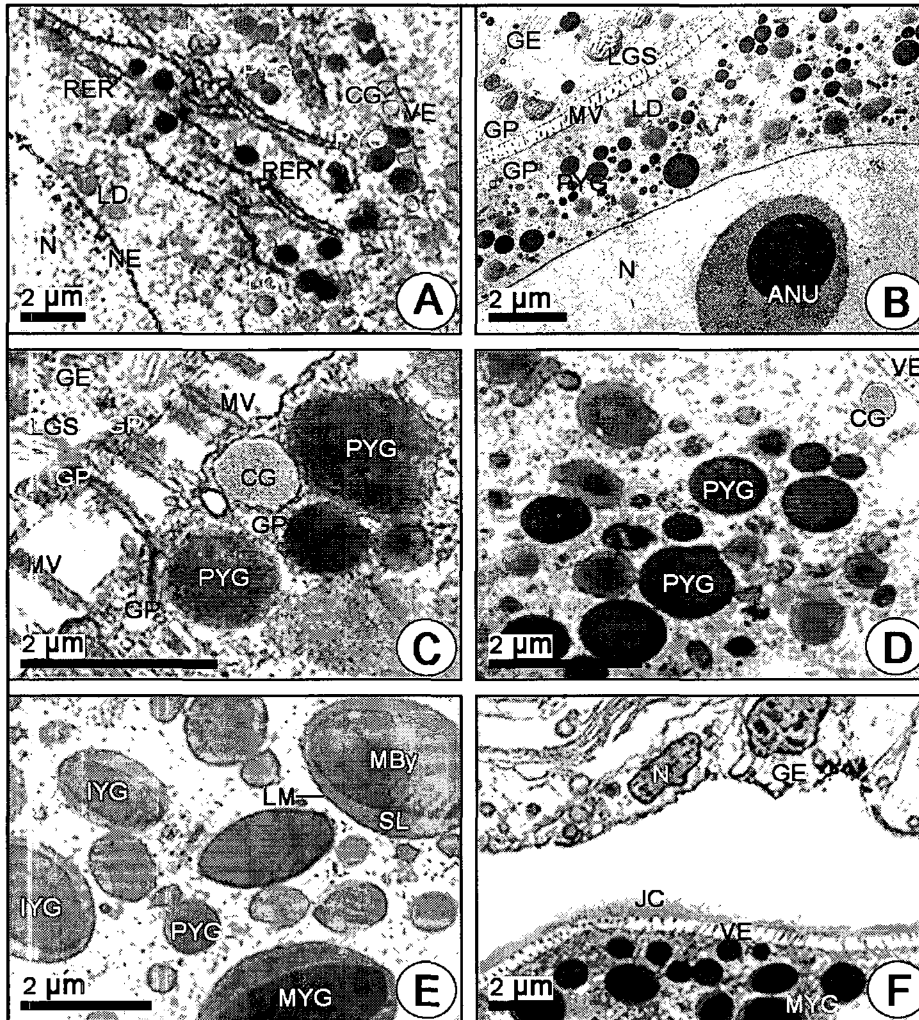

Fig. 4. Electron micrographs of late vitellogenic and mature oocytes during oogenesis of *Ruditapes philippinarum* (A - F). **A.** the late vitellogenic oocyte, with a number of proteid yolk granules among the lipid droplets, well-developed endoplasmic reticula, and several cortical granules near the vitelline envelope. **B.** A late vitellogenic oocyte attached to the germinal epithelium, with an amphinucleolus in the nucleus. **C.** A late vitellogenic oocyte, with a number of lipid granular substances and glycogen particles in the germinal epithelium passing into the ooplasm in the oocyte through the microvilli of the vitelline envelope. **D.** A late vitellogenic oocyte, with a number of proteid yolk granule in the perinuclear cytoplasm. **E.** A mature oocyte, with several immature and mature yolk granules. **F.** A mature oocyte, which was separated from the germinal epithelium, with a number of mature yolk granule and the vitelline envelope surrounded with the jelly coat.
Abbreviations: ANU, amphinucleolus; CG, cortical granule; GE, germinal epithelium; GP, glycogen particle; IYG, immature yolk granule; JC, jelly coat; LD, lipid droplet; LG, lipid granule; LGS, lipid granular substance; LM, limiting membrane; MBY, main body; MV, microvilli; MYG, mature yolk granule; N, nucleus; NE, nuclear envelope; PYG, proteid yolk granule; RER, rough endoplasmic reticulum; SL, superficial layer; VE, vitelline envelope.

layer near the vitelline envelope (Fig. 3F).

In the late vitellogenic oocyte, lipid droplets and lipid granules which occupy the area around the nuclear envelope, dispersed toward the cortical layer, and accumulation of cortical granules occurred in the cortical layer near the vitelline envelope autosynthetically. A number of proteid yolk granules, which were formed by well-developed rough endoplasmic reticula and cortical granules, appeared in the cortical layer (Fig. 4A). At this time, an amphinucleolus appeared in the nucleus of the late vitellogenic oocyte, and especially, exogenous electron dense lipid granular substances and lots of glycogen particles in the germinal epithelium were passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope (Figs. 4B, C). However, proteid yolk granules, which were formed by the cortical granules, endoplasmic reticula and glycogen particles (exogenous substance) in the cytoplasm, are dispersed from the cortical layer near the vitelline envelope to the perinuclear cytoplasm (Fig. 4D). And proteid yolk granules containing several different components were intermingled and became immature yolk granules in the oocyte (Fig. 4E).

Mature phase: In the mature phase, small immature yolk granules were fused to each other and became larger mature yolk granule. A mature oocyte was composed of three parts: 1) main body, 2) superficial layer, and 3) limiting membrane (Fig. 4E). At this phase, the tip of the microvilli, some of which bifurcate, protrude and extend just beyond the outer border of the vitelline envelope. The thick vitelline envelope of the mature oocyte was about 0.60 μm thick and covered with thick jelly coat. And then mature oocyte was separated from germinal epithelium (Fig. 4F).

3. Ovarian developmental stages and reproductive cycle

Based on morphological characteristics, differentiation of the germ cells and surrounding tissues during oogenesis, ovarian developmental stages can be divided into five successive stages: 1) early active stage, 2) late active stage, 3) ripe stage, 4) partially spawned stage, and 5) spent/inactive stage. Ovarian developmental stages of this species showed a periodicity through the year. Frequency of ovarian developmental stages of female clams was shown in

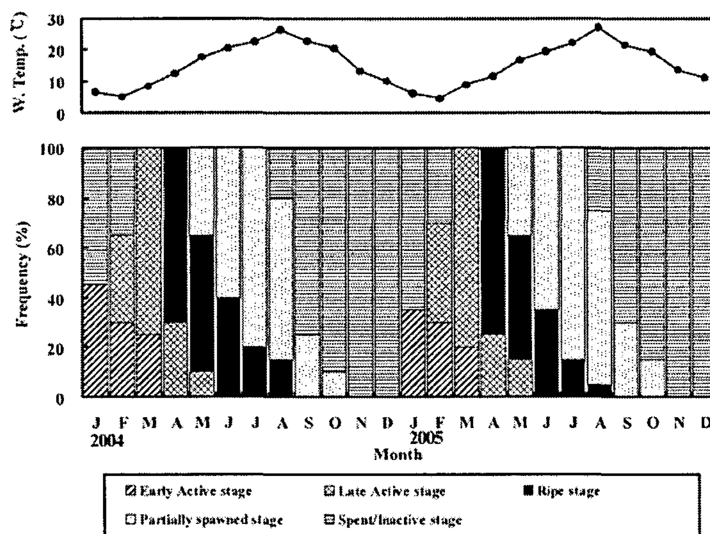

Fig. 5. Frequency of the ovarian developmental phases of female *Ruditapes philippinarum* and the mean seawater temperature, for two years from January 2004 through December 2005.

Fig. 5.

Early active stage: This stage was characterized by the expansion of the follicle and the appearance of oogonia, previtellogenic oocytes, undifferentiated mesenchymal tissues and eosinophilic granular cells along the follicular wall. No free oocytes were present in the lumen. At this time, the mean oogonium and previtellogenic oocyte were 10-11 μm and < 25 μm in diameter, respectively (Fig. 6A). Female individuals in the early active stage appeared from January to March in 2004 and 2005.

Late active stage: The undifferentiated mesenchymal tissues and eosinophilic granular cells in the follicle were gradually decreased, the early and late vitellogenic oocytes and a few free mature oocytes were present in the lumen of the follicle. More than

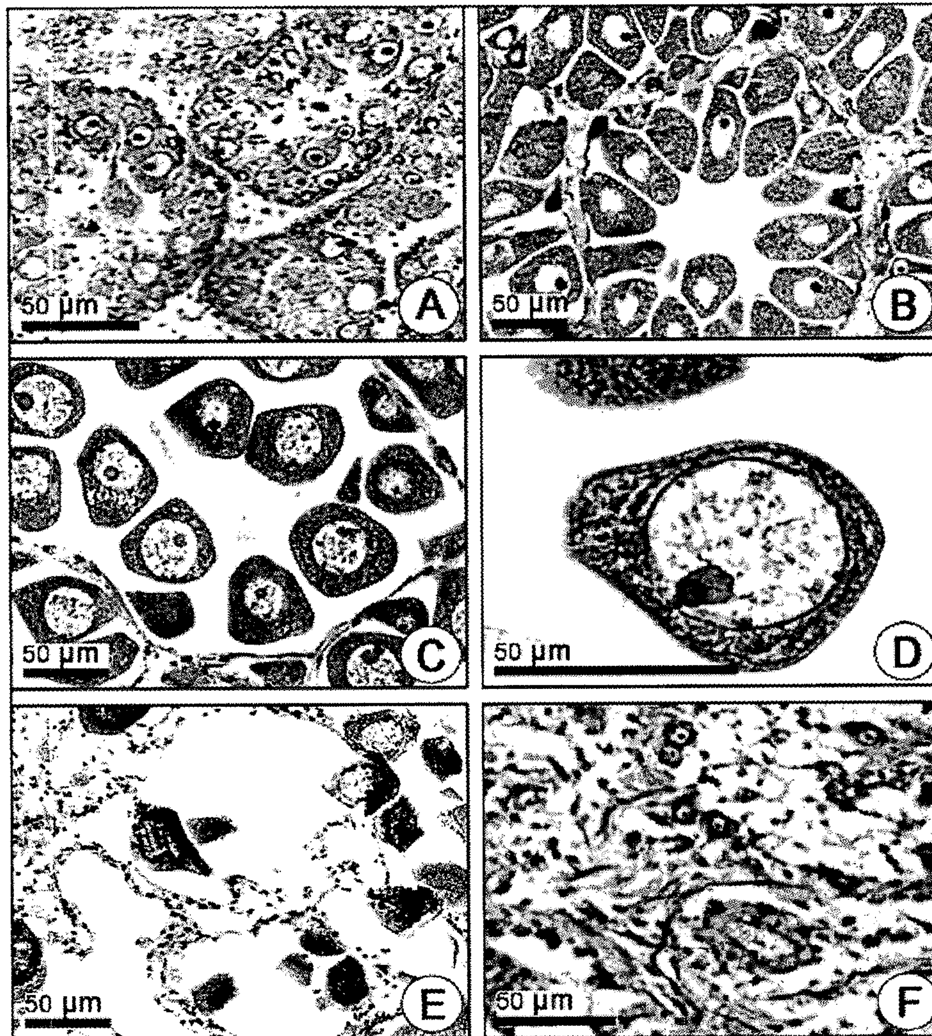

Fig. 6. Photomicrographs of the ovarian developmental phases of *Ruditapes philippinarum* (A - F). **A.** Transverse section of the oogenic follicles in the early active stage; **B.** Section of the follicles in the late active stage; **C.** Section of the follicles in the ripe stage; **D.** Section of a fully mature oocyte; **E.** Section of the follicles in the partially spawned stage; **F.** Section of the follicles in the spent and inactive stage. Scale bars = 50 µm.

half of the oocytes attached to the follicular wall, vitellogenic oocyte diameters were 40-50 μm. (Fig. 6B). The individuals in the late active stage were observed from February to May in 2004 and 2005.

Ripe stage: The ripe ovary exhibited distended follicles with mature and fully ripe oocytes. Undifferentiated mesenchymal cells and eosinophilic granular cells disappeared, and follicular wall was thin. Half or more than half of mature oocytes were free in the lumen of the follicle and the mean ripe oocyte diameter was 60-65 μm. in diameter. (Fig. 6C, D). The individuals in the ripe stage appeared from April to August in 2004 and 2005.

Partially spawned stage: The number of free mature oocytes in the follicle decreased because of discharging fully matured oocytes, and empty and ruptured follicles appeared. Some oocytes underwent cytolysis (Fig. 6E). Spawning in females occurred from late May to October, and peak spawning occurred between July and August in 2004 and 2005.

Spent/inactive stage: After spawning, follicles were shrunk and disorganized, and there was no sign of gonadal activity. Half or more than half of the follicles were empty. Follicles became contracted, and undischarged oocytes in the lumen underwent cytolysis. Thereafter, newly formed oogonia appeared among the connective tissues (Fig. 6F). The individuals in the spent/inactive stage appeared from August to February in 2004 and 2005.

DISCUSSION

It has been well-known that in the early vitellogenic oocytes of bivalves, the Golgi apparatus present in the perinuclear region is involved in lipid droplet formation, as in *Mytilus edulis* (Reverberi, 1971), *Mactra chinensis* (Chung, 1997) and *Mactra veneriformis* (Chung and Ryou, 2000). In the present study, a similar result was observed that the Golgi complex present in the region are involved in lipid droplet formation (referred as autosynthetic). Beside this finding, our electron microscope observation of early vitellogenic oocytes suggests that the mitochondria, endoplasmic reticula and glycogen particles in the perinuclear cytoplasm are also involved in the formation of lipid droplets in the cytoplasm autosynthetically.

Light microscope observations with the PAS stain showed a strong positive reaction at the site of the egg-stalk of the late active oocyte connected to the germinal epithelium (follicular wall). At this time, according to the results by electron microscope observation, electron dense lipid granular substances and glycogen particles in the germinal epithelium are passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope (referred as heterosynthetic). Therefore, we assume that some vitellogenic substances in the late vitellogenic oocyte are originated from exogenous substances in the germinal epithelium during vitellogenesis, as in *Mactra veneriformis* (Chung and Ryou, 2000) and *M. chinensis* (Chung, 1997).

Bottke *et al.* (1982) and Medina *et al.* (1986) reported that gastropod species, *Planorbarius corneus, Lymnaea stagnalis, Hypselodoris tricolor* and *Gordiva banyulensis* synthesize yolk by a combination of autosynthetic and heterosynthetic processes. Judging from our electron microscopical observations, it is assumed that *Ruditapes philippinarum* synthesize yolk by a combination of autosynthetic and heterosynthetic processes.

Associated with the formation of proteid yolk granules during vitellogenesis, some authors reported that the endoplasmic reticula and multivesicular bodies, as seen in the opisthobranchs *Hypselodoris tricolor* and *Godiva banyulensis* (Medina *et al.*, 1986), and another snails, *Physa acuta* (Terakado, 1974) and *Rapana venosa* (Chung *et al.*, 2002) are involved in the formation of proteid yolk granules in some gastropods (Taylor and Anderson, 1969). In the present study, we could not find multivesicular bodies which is formed by the modified mitochondria in the late vitellogenic oocyte, as seen in *Rapana venosa* (Chung *et al.*, 2002).

However, in the late vitellogenic oocyte of this species, especially, the proteid yolk granules appeared

near the endoplasmic reticula, mitochondria and cortical granules in the cortical layer. Therefore, it is assumed that the endoplasmic reticulum, mitochondria and cortical granules are involved in the formation of proteid yolk granules during vitellogenesis.

Many studies (Sastry, 1963, 1966, 1968, 1979; Sastry and Blake, 1971; Simpson, 1982; Chung *et al.,* 1991) have reported that gonadal development and maturation of bivalves is affected by environmental conditions, with exogeneous factors (water temperature, food organism, and day length) interaction with endogenous factors (neuronal and hormonal) inside the organism.

In the present study, *Ruditapes philippinarum* from Gomso Bay on the west coast of Korea initiated gonadal development during the late winter-early spring seasons when water temperatures were relatively low, and while chlorophyll-a level was high (Kim, 1999).

The gonadal phases were in the immature stage during the winter season because of lower temperatures and insufficient food organisms. Sastry (1966, 1968) contended that gonad growth and gametogenesis in *Argopecten irradians* took place under temperature conditions at which nutrient mobilization for the gonad occurred and described that temperature acted as a triggering stimulus for initiation of the oocyte growth phase. Therefore, we suggest that temperature and food availability required for active growth of oocytes at the beginning of oogenesis and for attaining maturity ultimately limit the annual period of gonad growth and gametogenesis in nature.

Gonadal development is an energy demanding process, as the mobilization of nutrients to the gonad is essential for gamete development. Although it is still unclear, gonadal development appears to depend on ingested food or stored reserves, or some combination of the two (Sastry, 1979; Barber, 1984). According to the results of Ministry of Maritime Affairs and Fisheries, Republic of Korea (2001), in Gomso Bay, food level (phytoplankton) was high in mid spring (April) and early summer (June). In the present study, thus, gonad growth and gametogenesis during mid spring (April) coincided with high food level. The highest food level that occurred in early summer will be necessary for oocyte maturity and spawning in *Ruditapes philippinarum.*

Investigations of natural reproductive cycle or spawning cycle are central not only to studies of population dynamics (*i.e.*, age determination and the recruitment period) but also to our understanding of biogeography and speciation. The reproductive cycle comprises the entire sequence of events from activation of the gonad through gametogenesis to spawning and the subsequent recession of the gonad (Chung, 1997). In nature there are considerable variations in the reproductive cycle of *Ruditapes philippinarum.* Intra-specific variations in the timing of spawning periods and the amount of produced gametogenic material vary with years and latitudinal gradient due to variations in environmental conditions influencing the reproductive process (Chung *et al.,* 1997).

Rand (1973) stated that breeding strategy varied with latitudinal gradient: *i.e.,* Northern climates were characterized by a single synchronous spawning every year, temperate climates by two spawning seasons and tropical ones by year-round spawning .

In case of different populations, there are some differences in the reproductive cycles of *Ruditapes philippinarum* in the other areas of the world; there is one spawning period in British Columbia, Canada (Quayle and Bourne, 1972), Hood Canal, Washington, USA (Holland and Chew, 1974), northern Japan (Yoshida, 1953), and Vostok Bay, northwestern part of the Sea of Japan (Ponurovsky and Yakovlev, 1992); while two in southern Japan (Tanaka, 1954; Ohba, 1959).

In the present study, this species has one spawning period as in the northern districts of Tokyo Bay, Japan. Therefore, it is assumed that the number of spawning frequencies in the same species varied with temperature-latitude.

REFERENCES

Baber, B.J. (1984) Reproductive energy metabolism in

the bay scallop, *Argopecten irradians concentricus* (Say). Ph.D. Thesis, University of South Florida, Tampa. 122 pp.

Bottke, W., Sinha, I. and Keil, I. (1982) Coated vesicle-mediated transport and deposition of vitellogenic ferritin in the rapid growth phase of snail oocytes. *Journal of Cell Science,* **53**: 173-191.

Choi, Y.M. (1987) The secondary production of the bivalve, *Tapes philippinarum,* in the shore of Sinsudo, Samcheonpo. Master Degree Thesis, National Fisheries University of Pusan. 45 pp. [in Korean].

Chung, E.Y., Lee, T.Y. and An, C.M. (1991) Sexual maturation of the venus clam, *Cyclina sinensis,* on the west coast of Korea. *Journal of Medical and Applied Malacology,* **3**: 125-136.

Chung, E.Y., Ryou, D.K. and Lee, J.H. (1994) Gonadal development, age and growth of the shortnecked clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae), on the coast of Kimje. *Korean Journal of Malacology,* **19**: 38-54.

Chung, E.Y. (1997) Ultrastuctural study of germ cell development and reproductive cycle of the hen clam, *Mactra chinensis* on the west coast of Korea. *Development and Reproduction,* **1**: 141-156.

Chung, E.Y. and Ryou, D.K. (2000) Gametogenesis and sexual maturation of the surf clam *Mactra veneriformis* on the west coast of Korea. *Malacologia,* **42**: 149-163.

Chung, E.Y., Kim, S.Y., Park, K.H. and Park, G.M. (2002) Sexual maturation, spawning, and deposition of the egg capsules of the female purple shell, *Rapana venosa* (Gastropoda: Muricidae). *Malacologia,* **44**: 241-257.

Goshima, S., Ide, N., Fujiyoshi, Y., Noda, T. and Nakao, S. (1996) Reproductive cycle and shell growth of transplanted Manila clam *Ruditapes philippinarum* in Saroma lagoon. *Nippon Suisan Gakkaish,* **62**: 195-200. [in Japanese].

Holland, D.A. and Chew, K.K. (1974) Reproductive cycle of the manila clam Washington. *Proceedings of National Shellfish Research,* **64**: 53-58.

Hur, Y.B. (1994) Comparative studies on the embryonic development and the growth of larvae of eight bivalve species. Master Degree Thesis, National Fisheries University of Pusan. 82 pp. [in Korean].

Kim, J.Y. (1999) Seasonal variation of the primary productivity in the vicinity of Chulpo Sea area. *Fisheries Science Research, Kunsan National University,* **14**: 177-122.

Kwon, O.K., Park, G.M. and Lee, J.S. (1993) Coloured Shells of Korea. 288 pp. Academy Publishing Co., Seoul. [in Korean].

Ohba, S. (1959) Ecological studies in the natural population of a clam, *Tapes japonica,* with special reference to seasonal variations in the size and structure of population and to individuals growth. *Biology of Journal of Okayama University,* **5**: 13-42.

Medina, A., Garcia, J.C., Moreno, F.J. and Lopez-Campos, J.L. (1986) Comparative studies on the histology of the ovotestis in *Hypselodoris tricolor* and *Godiva banyulensis* (Gastropoda, Opisthobranchia), with special reference to yolk formation. *Journal of Morphology,* **188**: 105-118

Ministry of Maritime Affairs and Fisheries, Republic of Korea (2001) Development of Optimal Technology for Sustaining Production in a Shellfish Farm, pp. 608-664. [in Korean].

Ponurovsky, S.K. and Yakovlev, Y.M. (1992) The reproductive biology of the Japanese littleneck, *Tapes philippinarum* (A. Adams & Reeve, 1850) (Bivalvia: Veneridae). *Journal of Shellfish Research,* **11**: 265-277.

Quayle, D.B. and Bourne, N. (1972) The clam fisheries of British Columbia. *Fisheries Research Board Canada Bulletin,* 179: 70-81.

Rand, W.M. (1973) A stochastic model of the temporal aspect of breeding strategies. *Journal of Theoretical Biology,* **40**: 337-351.

Redfern, P. (1974) Biology and distribution of the toheroa, *Paphies* (*Mesodesma*) *ventricosa* (Gray). *Fisheries Research,* **11**: 1-51.

Reverberi, G. (1971) *Mytilus* in experimental embryology of marine and freshwater invertebrates. *In*: Experimental Embryology of Marine and Fresh-water Invertebrates. (ed. by Reverberi, G.) pp. 175-187. North-Holland, Amsterdam, London.

Sagara, J. (1958) Artificial discharge of certain bivalves caused by treatment of sea water and by ingestion with NH_4OH. *Bulletin of Japanese Society Science Fisheries,* **23**: 505-510.

Sastry, A.N. (1963) Reproduction of the bay scallop, *Aequipecten irradians* Lamarck. Influence of temperature on maturation and spawning. *Biological Bulletin* (*Woods Hole*), **125**: 146-153.

Sastry, A.N. (1966) Temperature effects in reproduction of the bay scallop, *Aequipecten irradians* Lamarck. *Biological Bulletin* (*Woods Hole*), **130**: 118-134.

Sastry, A.N. (1968) Relationship among food, temperature and gonad development of the bay scallop, *Aequipecten irradians* Lamarck. *Physiology and Zoology,* **41**: 44-53.

Sastry, A.N. (1970) Reproductive physiological variation in latitudinally separated population of the bay scallop, *Aequipecten irradians* Lamarck. *Biological Bulletin* (*Woods Hole*), **138**: 56-65.

Sastry, A.N. (1979) Pelecypoda (excluding Ostreidae). *In*: Reproduction of Marine Invertebrates, Volume V, Molluscs: Pelecypods and Lesser Classes. (ed. by Giese, A.C. and Pearse, J.S.) pp. 113-292. Academic Press, New York.

Sastry, A.N. and Blake, N.J. (1971) Regulation of gonad development in the bay scallop, *Aequipecten irradians* Lamarck. *Biological Bulletin* (*Woods Hole*), **140**: 274-282.

Simpson, R.D. (1982) Reproduction and lipids in the

sub-Antarctic limpet *Nacella* (*Patinigera*) *macquariensis* Finlay, 1927. *Journal of Experiment Biology and Ecology,* **56**: 33-48.

Taylor, G.T. and Anderson, E. (1969) Cytochemical and fine structural analysis of oogenesis in the gastropod, *Ilyanasa obsolete. Journal of Morphology,* **129**: 211-248.

Tanaka, Y. (1954) Spawning season of important bivalves in Ariake Bay-III. *Venerupis semidecussata. Bulletin of Japanese Society Science Fisheries,* **19**: 1165-1167. [in Japanese with English abstract]

Terakado, K. (1974) Origin of yolk granules and their development in the snail, *Physa acuta. Journal of Electron Microscopy,* **23**: 99-106.

Toba, M., Natsume, Y. and Yamakaw, H. (1993) Reproductive cycles of Manila clam collected from Funabashi waters, Tokyo Bay. *Nippon Suisan Gakkaishi,* **59**: 15-22 [in Japanese].

Toba, M and Miyama, Y. (1994) Relationship of size to gonadal maturation and spawning in artificially conditioned Manila clams. *Nippon Suisan Gakkaishi,* **60**: 173-178 [in Japanese].

Toba, M. and Miyama, Y. (1995) Influence of temperature on the sexual maturation in Manila clam, *Ruditapes philippinarum. Suisanzoshoku,* **43**: 305-314.

Tsuji, S., Munekiyo, M., Itani, M. and Douke, A. (1994) Reproductive cycle of a Manila clam in Maizuru Bay. *Bulletin of Kyoto Ocean Center.* pp. 1-9. [in Japanese]

Yoo, J.S. (1976) Korean Shells in Colour. 70 pp. Ilgisa, Seoul. [in Korean]

Yoon, S.B. (1992) Population dynamics of the shortnecked clam, *Tapes philippinarum* in An-Jong, Tong-Young. Master Degree Thesis, National Fisheries University of Pusan, 34 pp. [in Korean]

Yoshida, H. (1953) Studies on larvae and young shells of industrial bivalves in Japan. *Journal of Shimonoseki College Fisheries,* **3**: 1-106 [in Japanese].